UCSF iGEM 2015

Master Gene List

Note: Old primers, we have newer ones with much higher numbers.

Gene | FW Primer | Rev Primer | Length (bp) ---------- | --------- | ---------- | ---------- pTEF1 | 113 | 114 | 450 pTEF1 (m3) | ? | 114 | 450 pTEF1 (m6) | 116 | 114 | 450 pTEF1 (m7) | 117 | 114 | 450 pTEF1 (m10) | 118 | 114 | 450 pBAR1 | 120 | 119 | 350 BAR1 | ? | 93 | 1,700

Master Plasmid and Yeast List

Name | Promoter | Gene | Plasmid | Length (bp) ---------- | ---------- | ---------- | ---------- | ---------- pGEM 45 | pTEF1(m10) | BAR1 | pJW608 | 9000 | 450 pGEM 58 | pTEF1 | ?? | 450 pGEM 59 | pTEF1(m3) | ?? | 450 pGEM 60 | pTEF1(m6) | ?? | 450 pGEM 61 | pTEF1(m7) | ?? | 450 yGEM 128 | 120 | ?? | 350 yGEM 1 | ? | GFP | 1,700

6/8/15 - Day 1 Bootcamp

1. Cloning with Samantha
    - Bar1 - sticks to protein's cell walls, protease enzyme
    - pTEF1(m3)(m3)(+Apa1)FW primer
    - pTEF1(m3)(m3)(+Xho1)RV primer
    - Followed Primer Design protocol

    Make sure to dilute primers to 10 uM from 100 uM stock primers
    Add 10uL of 100uM stock to 90uL of H2O

2. PCR for Constitutive Primers
    Promoter and Bar1 both placed in PCR thermocycler overnight
    Primer stock solutions placed in Samantha's freezer box

6/9/15 - Day 2 Bootcamp

1. Ran PCR products in gel - worked!
    1. 1 uL loading dye (6x)
    2. 5 uL PCR product
    3. Vortex
    4. Pour small 35 uL gel
        - Lane 1- gel ladder (7 uL)
        - Lane 2- pTEF1(m3)(m3) (5 uL)
        - Lane 3- Bar1 (5 uL)

Always run at 100V!

2. Plasmid Digest

--- Apa1 -- promoter -- Xho1-- Bar1 -- Not1 -- Plasmid --
Note: Always keep enzymes on ice!

- pTEF1(m3) and plasmid (PJW608) incubated at room temperature for an hour before adding the terminator primers
- Ran 3 PCR tubes:
  - pTEF1(m3)
  - Bar1
  - pJW608 (plasmid)

6/10/15 - Day 3 Bootcamp

1. Ran PCR products in gel

- Lane 1 - ladder (10 uL)
- Lane 2 - pTEF1(m3) (40 uL)
- Lane 3 - pTEF1(m3) (10 uL)
- Lane 4 - BAR1 (40 uL)
- Lane 5 - BAR1 (10 uL)
- Lane 6 - PJW608 (36 uL)

2. Gel Extraction
Follow QIAquick Gel Extraction Kit using a Microcentrifuge Protocol

1. Gel Weights

- blank microcentrifuge tube: 1044.9 mg
- pTEF1(m3): 201.9 mg
- Bar1: 355.1 mg
- plasmid (PJW608) : 1243.0 mg

2. Add 3 volumes of buffer QG to 1 volume of gel (100 mg ~ 100 uL)

Vector | QG Buffer Added | --------- | ---------- | pTEF1(m3) | 300 uL | BAR1 | 600 uL | plasmid | 300 uL |

3. Add 1 gel volume of isopropanol to sample and mix

Vector | Isopropanol Added | --------- | ---------- | pTEF1(m3) | 200 uL | BAR1 | 355 uL | plasmid | 198 uL |

4. Elute DNA with 30 uL of water

- Let sit for 1 minutes, centrifuge for 1 min.
- *Note: Place directly at the the center of the spin column cloth*
  - Otherwise will not properly elute and will combine ethanol

5. Nanodrop

        - Blank with ddH2O
        - Negative nucleic acid concentration = potential ethanol contamination
        - Should usually see peak on graph, numbers around 30 to 1000
3. Plasmid Ligation - using 2 kb

    - Vector DNA: 2 uL (50 ng)
    - Insert: 37.5 ng x 2 = 75 ng
        - 75 ng / 15.44 ng/uL = 5 uL of Bar1
        - 10 uL pTEF1(m3)
        - 0 uL H2O
        - 2 uL DNA Ligase buffer
        - 1 uL DNA ligase

(good negative control would be if you don't put in the insert, ligate plasmid on its own = measures background)

1. E.Coli Transformation using New England BioLabs High Efficiency Transformation Protocol (C2987H/C2987I) Notes:

    - Competent E.Coli cells are in the -80 freezer, second shelf, middle box, 2nd row. The tubes are labeled with the E.Coli number.
    - When adding 5 uL of plasmid DNA to cell mixture, swirl with pipet tip. DON'T PIPET UP AND DOWN!
    - SOC media is easily contaminated because of high sugar content; be cautious to prevent contamination!
2. E.Coli Plating

    1. Get warmed plates from incubator
    2. Labeled plates "pTEF1(m3) - BAR1 Ligation" + initials + date
        - Plate #1 - 1x 100uL
        - Plate #2 - 8x 100uL of resuspended ligated cells
            - Pellet at 7-8000 rpm for 30 seconds
            - Get 100 uL out, dump the rest and resuspend cells
            - Put 100 uL of resuspended cells in the 8x labeled plate
        - Shake plates with beads back and forth on table, not in the air

6/11/15 - Day 4 Bootcamp

1. Colony PCR for Screening E.coli

    1. Picked a single colony to plate, did this for 6 tubes
    2. PCR "Master Mix" (7x)
        - 2x GoTaq Green PCR Mix - 70 uL
        - 10 uM pTEF1(m3) FW primer - 7 uL
        - 10 uM Primer 93 RV - 7 uL
        - water - 21 uL
    3. Added 15 uL of 7x Master Mix to 5 uL colony-filled tubes for total of 20 uL material.
    4. Put in thermocycler

2. E.Coli Gel PCR
    - Lanes 2-7 - Samantha and I's E.coli colony PCR - did not show bands
3. Culture Transformed E.coli in LB

6/12/15 - Day 5 Bootcamp

6/16/15

Bar-1 Constructs for iGEM Project: setting parameters for Bar-1 as T-reg analogs

```
A. Sent in CPL1(4)-Bar1 and CPL1(5)-Bar1 for sequencing to Quintara
    Came back with an empty plasmid PJW608
    Renamed tube "PJW608 Sequenced" and placed in Parts&Plasmids 2014 box
```

6/17/15

1. Promoter PCR
    - Followed Promoter PCR Reaction Protocol to re-PCR all pTEF1(m3) mutants
    - Could not find pGEM 13 - pTEF1(m3)

    Tube Label | Template | Promoter | Primers ---------- | -------- | -------- | ------- A | pGEM 17 | pTEF1 | 113 FW/114 RV B | pGEM 19 | M6 | 116 FW/114 RV C | pGEM 20 | M7 | 117 FW/114 RV D | pGEM 16 | M10 | 118 FW/114 RV

        1. Ran promoters PCR products in gel - A and D did not work, B and C did

6/18/15

1. Check sequencing for "pGEM13 + rtTA3" and "pGEM13 + rtTA4" -- sequences matched and tubes contain pGEM18.
    - Relabeled tube "pGEM18 sequenced" and placed in Parts&Plasmids box
2. Ran gel for PCR products (10 uL of loading dye/50 uL of product)

1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 ----- | ------ | ------ | -- | -------- | -------- | -- | ------ | ------- ladder | BAR1-A | BAR1-B | x | pGEM13-1 | pGEM13-2 | x | A redo | D redo

1. Isolate pBAR1 from Cadherin
    - Followed Zymolase Yeast Plasmid Miniprep Protocol
2. PCR pBAR1

```
Materials
    template DNA (Zymolase Cadherin) - 5 uL
```

```
    primer 119 - 2.5 uL
    primer 120 - 2.5 uL
    2x Fusion Master Mix - 25 uL
    water - 15 uL
    total - 50 uL

Ran under Phusion protocol on PCR machine
```

6/19/15

1. PCR purified pBAR1, followed PCR Purification Protocol
2. Ran gel to verify pBAR1 presence
    - Lane 1 - DNA ladder
    - Lane 2 - pBAR1-A
    - Lane 3 - pBAR1-B

   No pBAR1 appearing in gel, very streaky
   Kara thinks it might be because the promoters are too large to show up on the gel
3. Digested pJW608 with Xho1 and Not1
4. Digested BAR1 with Xho1 and Not1
5. Re-PCR pTEF1(m3) with EC template, run in gel

6/22/15

1. Ran pTEF1(m3) PCR product in gel, worked!
2. Gel Purification of Digested Bar1 and Plasmid
    - Used QIAquick PCR Purification Kit
    - 1:5 Buffer PB:digest pJW608 digested (30 uL), added 6 uL Buffer PB Bar1 digested (56 uL), added 11 uL Buffer PB

   Vector | Quantity Digested [uL] | Buffer PB [uL] ------ | :----------: | ---------- | pJW608 | 30 | 6 | BAR1 | 56 | 11 |
3. Phosphatase'd then --> Ligation

   Material | Negative Control Ligase | BAR1 Ligase ---------- | :----------: | ---------- | pJW608 | 2 uL | 2 uL | BAR1 | 0 uL | 1 uL | Ligase | 1 uL | 1 uL | Ligase Buffer | 1 uL | 1 uL | H2O | 16 uL | 15 uL | **Total** | 20 uL | 20 uL |

6/23/15

1. Transformed BAR1 ligase and negative control
    - Used New England BioLabs High Efficiency Transformation Protocol (C2987H/C2987I)
2. Plated 4 groups of E. Coli cells
    - 1X BAR1 NEF5-alpha
    - 8X BAR1 NEF5-alpha
    - 1X Negative Control
    - 8X Negative Control

6/24/15

1. Collected 6 colonies each from 1X BAR1 and 1X Negative Control plates in 25 uL of H2O.
2. Pipetted 5uL of each colony sample into PCR tube
3. Ran E.Coli PCR
4. Loaded colonies into gel, ran at 100V for 20 minutes
   insert gel picture here
5. Bands appeared for Colonies 3, 4, 6 of 1X BAR1 and for one column
   from 1X Negative Control

6/25/15

1. MiniPrep'd Colonies 3, 4, 6 from 1X BAR1 plate that were PCR'd
   and then ran on a gel yesterday (6/24)
2. Nanodropped Colonies 3, 4, 6 with these resuls:

   | Colony | Concentration [ng/uL] | |:---------- |:----------:| | A (prev 3) | 466 | | B (prev 4) | 326 | | C (prev 6) | 414 |

3. Set up ligation of more BAR1 in anticipation of failed colony PCR

    - Ligases in freezer for possible future use

6/26/15

1. DNA Sequencing for Colony B worked
   A. Colony C had a point mutation so it will not be used
   B. Labeled Colony B as pGEM 45 and placed in plasmid box
2. Re-PCRing pTEF promoters and mutants

    - Threw away previously PCR'd promoters
    - **Already have A and B PCR'd** but **need to *DNA purify B***

   Tube Label | Template | Promoter | FW Primer | RV Primer | ---------- | -------- | -------- | --------- | --------- | **A** | pGEM17 | pTEF1 | 113 FW | 114 RV | **B** | pGEM13 | M3 | 115 FW | 114 RV | C | pGEM19 | M6 | 116 FW | 114 RV | D | pGEM20 | M7 | 117 FW | 114 RV | E | pGEM16 | M10 | 118 FW | 114 RV |

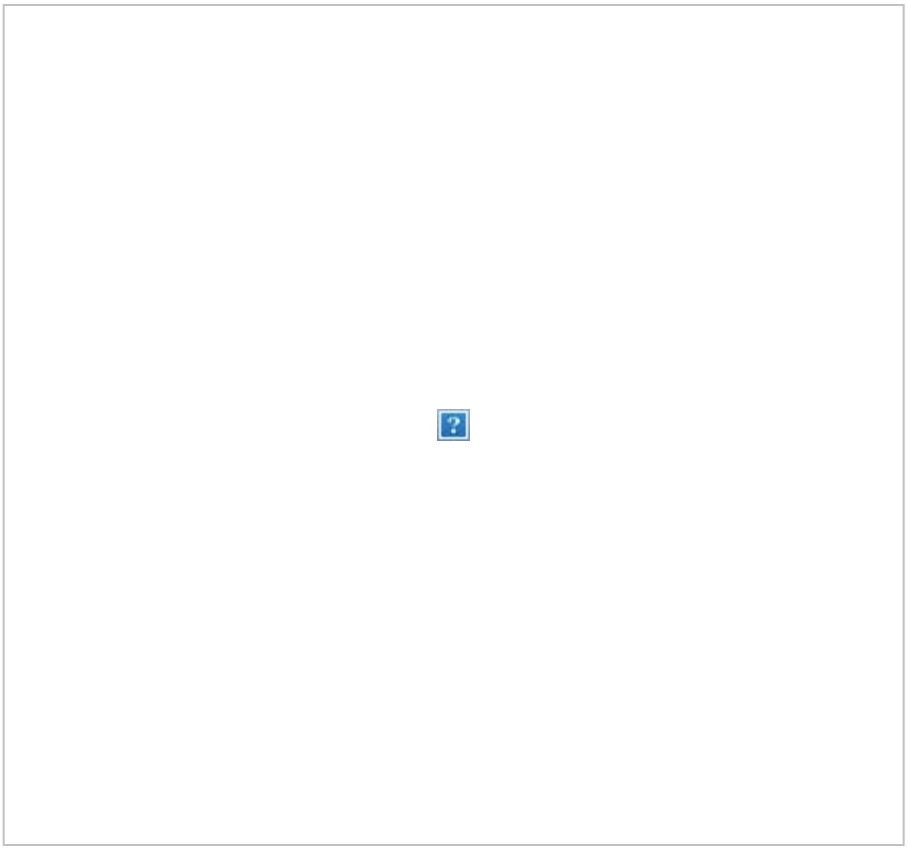
3. Dyed and loaded 5 uL of each PCR product in 1.5X agarose gel
    - Loaded C, D, E in last three columns before ladder respectively
    - SUCCESS

6/29/15

1. Need to digest pTEF (incl mutants) and plasmids
    - Running gel to confirm pTEF1
    - Also digesting suspected pTEF1 while running gel
2. Digesting pTEF promoters
    - 5 uL cutsmart
    - 0.5 uL Apa1 --> incubate at room temp for 1 hour
    - 0.5 uL Xho1 --> incubate at 37C overnight
3. Forgot to PCR purify pTEF1(m3) mutant
    - **Digest this tomorrow**

pTEF1 and pTEF1(m3) gel runs - **Success**! ~400 bp.

- Lane 1: DNA Ladder
- Lane 3: pTEF1
- Lane 4: pTEF1(m3)



6/30/15

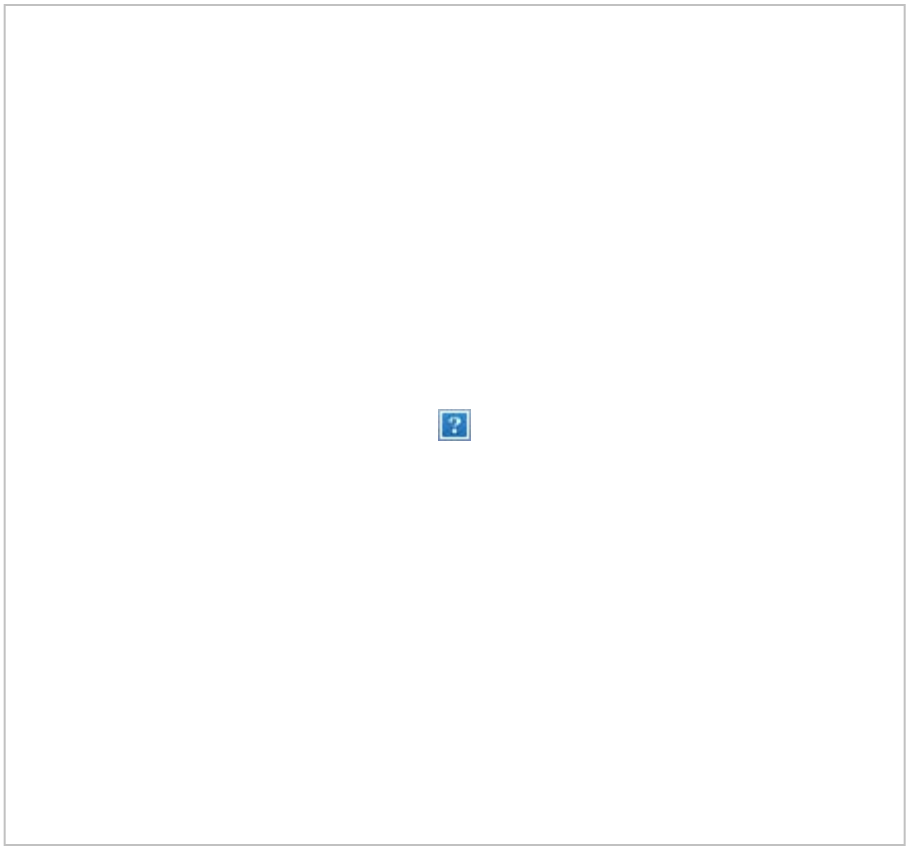
1. Digest pTEF1(m3)
    - 0.5 uL Apa1 --> incubate at room temp for 1 hour
    - 0.5 uL Xho1 --> incubate at 37C for 1 hour
2. Digest plasmid
    - 0.5 uL Apa1 --> incubate at room temp for 1 hour
    - 0.5 uL **Not1** --> incubate at 37C for 1 hour (done at 12pm)

DO NOT USE NOT1 HERE. THIS SHOULD HAVE BEEN XHO1.

1. Gel purify digested promoters and plasmid?

    - Run gel (1% agarose), use large lanes
2. Gel run was **terrible**, everything failed

    - Promoters showed up past 400bp, possible primer dimer.
    - Plasmid appears as a huge streak, probably did not cut
        - Because **wrong 2nd enzyme** was used.



Lanes:

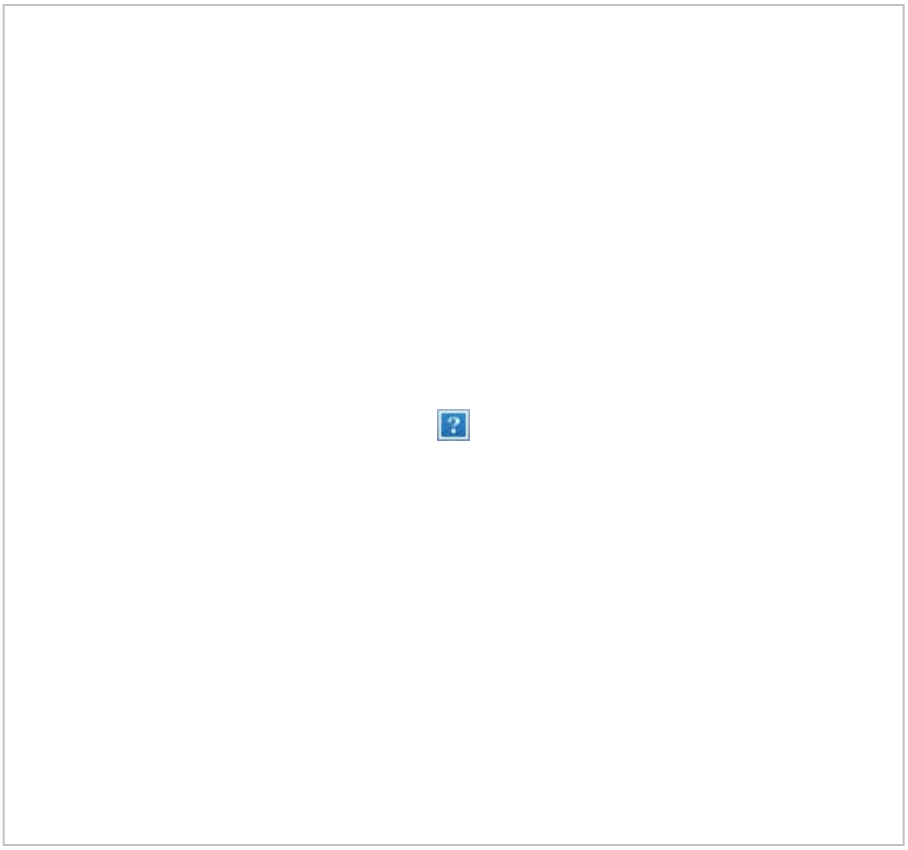
- Lane 1: ladder
- Lane 3: pTEF1
- Lane 5: pTEF1(m3)
- Lane 7: pTEF1(m6)
- Lane 9: pTEF1(m7)
- Lane 11: pTEF1(m10)

- Lane 13: BAR1 digested plasmid
- Starting new PCR of promoters.

7/1/15

1. Running 1% agarose gel
    - Lane 1: 2-log DNA Ladder
    - Lane 3: pTEF1
    - Lane 5: pTEF1(m3)
    - Lane 7: pTEF1(m6)
    - Lane 9: pTEF1(m7)
    - Lane 11: pTEF1(m10)

Everything worked except pTEF1, see gel below

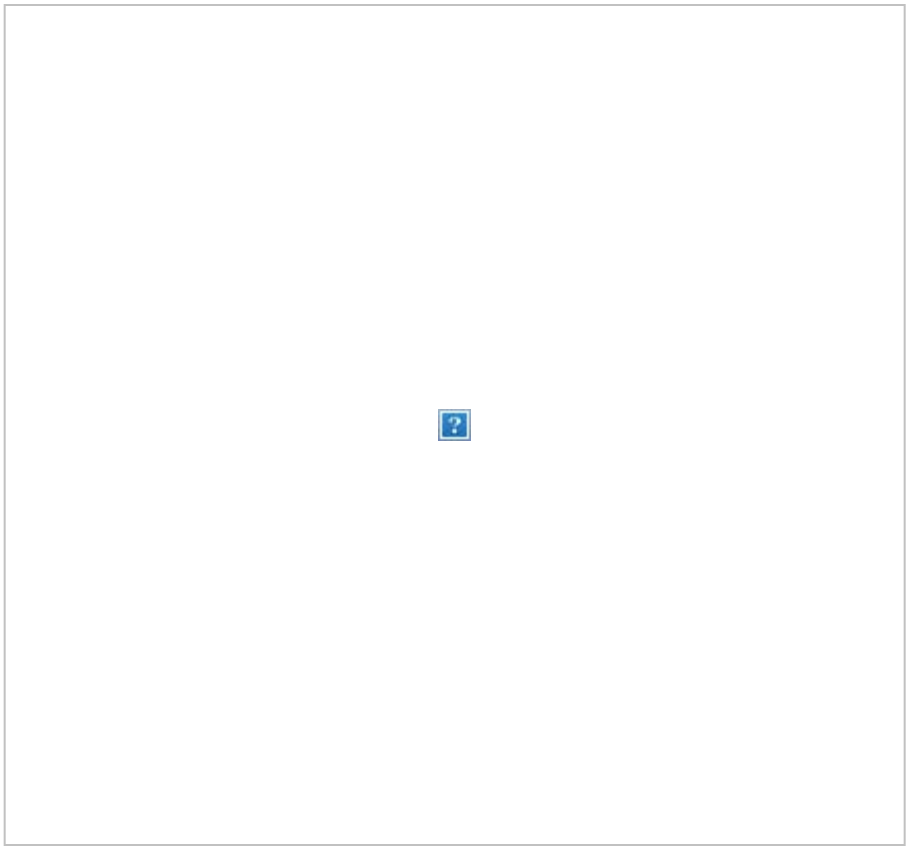


7/2/15

1. PCR pTEF1
    - Running pTEF1 from Erika's box + pGEM17
    - Gel'd. Failed. Again. Image not shown, but it was messy.
2. Run gel of digested promoters and plasmid

    - Discovered **wrong restriction enzyme** was being used on plasmid.

- Re-digesting plasmid, otherwise promoters came out well. See below Lanes:
- 2log DNA Ladder
- Skipped
- BAR1 Plasmid
- Skipped
- pTEF1(m3)
- pTEF1(m3) overflow
- pTEF1(m6)
- pTEF1(m6) overflow
- pTEF1(m7)
- pTEF1(m7) overflow
- pTEF1(m10)
- pTEF1(m10) overflow
- Skipped
- 2log DNA Ladder



3. Gel extracted, Nanodropped, found following concentrations

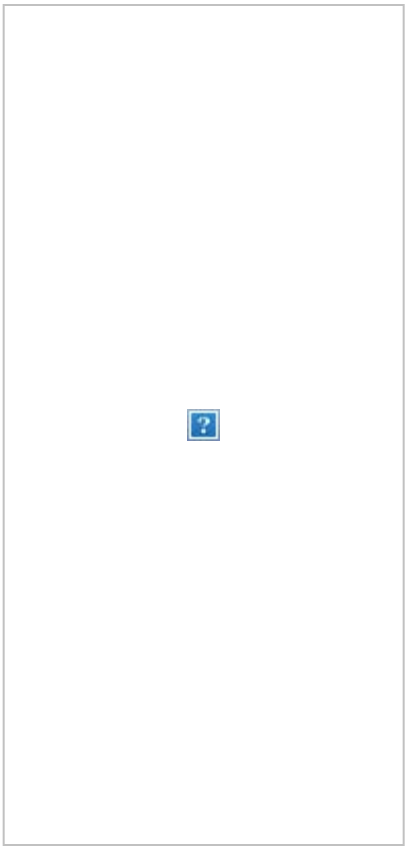
pTEF Mutant	Gel weight (mg)	Buffer QG Added (3mL:1)	Nanodrop concentration (ng/uL)
pTEF1 m3	647	1950	79.93

pTEF1 m6	569	1710	63.22
pTEF1 m7	387	1160	74.01
pTEF1 m10	808	2430	59.07

4. Extract pBAR1 from Yeast Cadherin cells

5. Digesting plasmid pGEM45 again
    - 20 uL plasmid
    - 2.5 uL CutSmart
    - 0.5 uL Apa1 --> Incubate 1 hour at room temperature
    - 0.5 uL Xho1 --> Incubate 1 hour at 37C

Plasmid ~9kbp, shows up on right side



7/6/15

1. Somehow need to get pTEF1

    - Try with GoTaq?
        - Running PCR using GoTaq --> pGEM17 and Erika's pTEF1
        - **Did not amplify**, the gel showed nothing
            - Erika is running one more pTEF1 (see below pt4)
    - Extract from yeast we are getting pBAR1 from

        - Endogenous promoter for TEF1 in the yeast genome
2. BAR1 plasmid is digested **[225.1 ng/uL]**, ligate pTEF1 mutants with plasmid

    - **DILUTION**: BAR1 diluted 3X (3uL BAR1, 6 uL H2O)
        - Technically have 100% yield... improbable but not impossible
    - **DILUTION**: pTEF1 mutants diluted 5X (4uL H2O, 1uL promoter)

Material | Negative Control Ligase | pTEF Ligase ---------- | :----------: | ---------- | diluted pGEM45| 1 uL | 1 uL | diluted promoter | 0 uL | 1 uL | Ligase | 1 uL | 1 uL | Ligase Buffer | 2 uL | 2 uL | H2O | 16 uL | 15 uL | **Total** | 20 uL | 20 uL |

1. Erika worked with yeast genome to extract pBAR1 and pTEF1

2. Gellin'

    - Gel Lanes (skipping lanes):
        - Lane 1: BAR1
        - Lane 2: pBAR1
        - Lane 3: pTEF1
        - Lane 4: 2log DNA Ladder

IMAGE

7/7/15

1. Colony PCR
    - Running 4 colonies of each
    - Gel running, need to image
2. Gel purify/extract BAR1 plasmid
    - Running gel
    - No longer extracting, it's ugly.
3. Rerun PCR for pTEF1 (pTEF1 as backbone)
    - Did not do, Erika digested, can do tomorrow
4. Ligate pTEF1 mutants (if Colony PCR doesn't work)
    - Not doing this yet, but will need to be done tomorrow?
    - Colony PCR worked, do not need to do this
5. Digest pBAR1 and pTEF1 (overnight) --> gel extract. Ligate into cut plasmid.
    - These are digesting overnight.
6. Transforming pGEM45 --> C29087 Cells to make more pGEM45
    1. Add 25uL cells to 0.5uL pGEM45
    2. Incubate on ice for 30min
    3. Heat shock at 42C for 45 seconds
    4. Incubate on ice for 2min
    5. Add 250uL SOC
    6. Incubate for 1 hour
    7. Plate on LB+carb ---> Currently incubating at 37C --> overnight

Snapcaps and Falcon Tubes.

Colony PCR Gel Lanes Today: From right side: (bold indicates positive)

1. pTEF1(m10) Colony 3
2. **pTEF1(m10) Colony 2**
3. **pTEF1(m10) Colony 1**
4. **pTEF1(m7) Colony 3**
5. **pTEF1(m7) Colony 2**
6. pTEF1(m7) Colony 1
7. **pTEF1(m6) Colony 4**
8. **pTEF1(m6) Colony 3**
9. pTEF1(m6) Colony 2
10. pTEF1(m6) Colony 1
11. **pTEF1(m3) Colony 4**
12. **pTEF1(m3) Colony 3**
13. pTEF1(m3) Colony 2

image

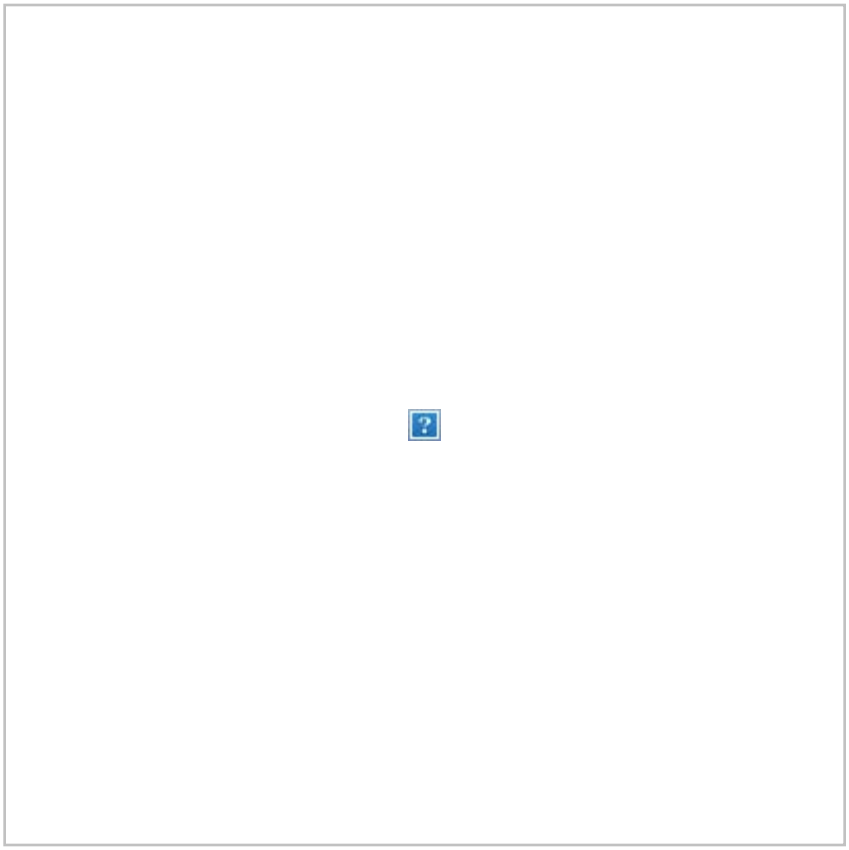
- Colonies were suspended in media, put on shaker in 37C incubator room

7/8/15

1. Ran gel of digested pTEF1 and pBAR1

Lanes:

1. 2log DNA Ladder
2. Blank (we think ladder leaked here)
3. pBAR1 Digested
4. pBAR1 Digested
5. Blank
6. pTEF1 Digested (these lanes we see bands around 500+?)
7. pTEF1 Digested



pBAR1 ~ 350bp, **pTEF1** ~ 450bp

2. Gel extract pBAR1 and pTEF1

3.. Harvested 2 colonies from pGEM45 + pJW608 plated on LB + Carb

- Suspended in liquid colonies of 5 mL LB+Carb, in incubator at 37C
- Will MiniPrep tomorrow

4. MiniPrep/Sequence pTEF1(mutants)

- Sent for sequencing

7/9/15

1. MiniPrep and sequence pGEM45

- Sent for sequencing
- Digested and phosphotase treated
- Ran gel and extracted - put in tube to be purified tomorrow

2. Sequenced pTEF1 mutants, only m10 worked

- Sent other 4 colonies of mutants for sequencing

- Added 4.16uL of pTEF1(m10) to PME digest
- Ran Yeast Transformation Protocol
    - Now incubating in 30C for a few days

3. Transforming pTEF1(m10) into yeast

```
Before Transforming:
1. Take 500 uL of overnight stock
2. Put into glass culture tube, add 9.5 mL of YPD
3. Incubate in 30C for 3 hours
4. PME Digest (below) (1 hour)
5. Prep ssDNA (20 min)

PME Digest:
- 2000ng DNA (pTEF1(m10))
- 0.5uL of PME1 enzyme
- 1 uL CutSmart
- Add H2O up to 10uL
- Incubate for 1 hour at 37C

Salmon Sperm DNA Prep:
- Take tube from freezer
- Boil (95C) in PCR machine for 10 min
- Put on ice for ten minutes
```

7/10/15

1. Sequencing from yesterday:

- pGEM45 accurately transformed
- pTEF1(m6) and pTEF1(m7) have BAR1 but no promoter

2. Extract pGEM45 from gel ran yesterday

- Extracted, labeled as pGEM 45 gel extracted, in freezer box.
- Used pGEM45 to ligate in next step

3. Miniprep colony with pTEF1(m3)

- Miniprepped
- Sent for sequencing

4. Ligate pTEF1 and pBAR1 into pGEM45

- Set up 3 ligations
    1. Negative control (no promoters)

2. pTEF1 Ligase (Used A labeled tube)
3. pBAR1 Ligase (Use A labeled tube)

- Diluted promoters:
  - pTEF1 is not diluted, we need 50ng of vector to 7.5ng of insert
    - Using pTEF1(A) labeled in box [16ng/uL]
    - Adding 0.5 uL --> 8ng insert
  - pBAR1 is diluted. Need 50ng of vector to 5.8ng of insert
    - Using pBAR1(A) labeled in box [41.83ng/uL]
    - 3x dilution: 1uL of pBAR1(A) + 3 uL of H2O --> 10.45ng/uL
    - Add 0.6 uL of this dilution --> 6.27ng of insert
  - pGEM45 is not diluted, we need 50ng of vector
    - Using pGEM45 digest labeled in box [29.86ng/uL]
    - Adding 2 uL --> 60ng

Material | Negative Control Ligase | pTEF1 Ligase | pBAR1 Ligase ---------- | :----------: | ---------- | ---------- | pGEM45 | 1 uL | 2 uL | 2 uL | diluted promoter | 0 uL | 0.5 uL | 0.6 uL | Ligase | 1 uL | 1 uL | 1 uL | Ligase Buffer | 2 uL | 2 uL | 2 uL | H2O | 16 uL | 14.5 uL | 14.4 uL | **Total** | 20 uL | 20 uL | 20 uL |

7/13/15

1. Check replica plated pGEM46

- Checked, looks good, many good sized colonies
- Patch plated 6 colonies and placed in 30C incubator

2. Colony PCR pGEM46 in Yeast Plate

1. Use primers from Kara: Primers 87 and 91
2. Set up Colony PCR --> GoTaq
   - **Zymolase treat first** (chews up holes to allow us to PCR)
   - Product should be ~945bp

3. Checked sequencing of pTEF1(m3) in pGEM45

1. Sequence came back negative, only had BAR1

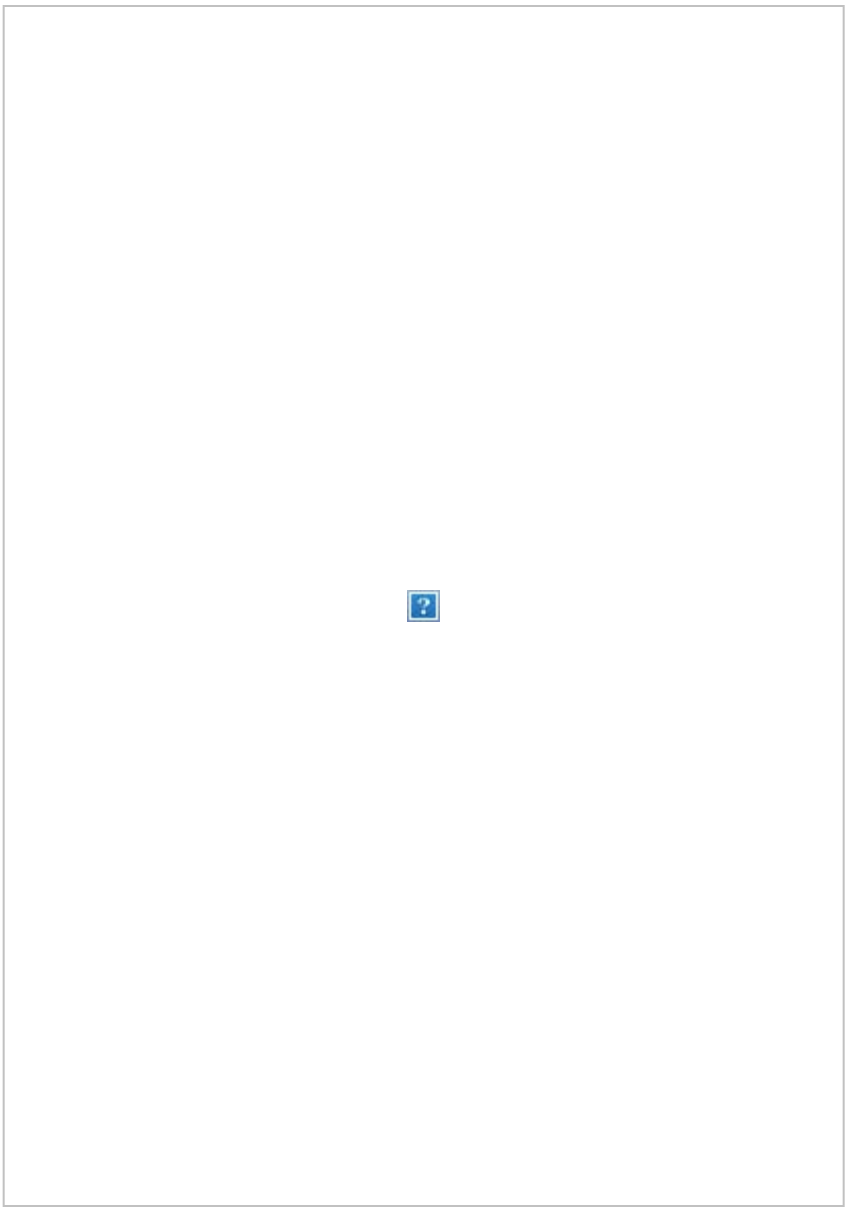
7/14/15

1. Run Colony PCR of yeast transformed pGEM46 gel

- Looking for ~950bp from primer/yeast binding site
- Lanes:

1. 2log DNA Ladder

2. Colony 1
3. Colony 2
4. Colony 3 (split between lanes due to spill)
5. Colony 3 (split between lanes due to spill)
6. Colony 4
7. Colony 5



- Chose Colony 1 (best looking band) to process

2. Prepped Colony 1 for glycerol stock

- Restreaked Colony 1 on new plate
- Overnight culture cells in glycerol stock

3. Redigest m3, m6, m7 for ligation with pGEM45

- Use Apa1 & Xho1 to digest

- Once done, will PCR purify, nanodrop, and ligate

4. Transform pTEF1 & pBAR1 into E. Coli

- Plated on LB + Carb for overnight
- Also plated a negative control

7/15/15

1. Colony PCR pTEF1, pBAR1 & Negative Control transformation from yesterday

- O/N culture positive PCR bands --> **none**

Gel did not work, primer dimer

- Picking 4 new colonies and re-PCRing
  - pTEF1 Col 5, 6, 7, 8
  - pBAR1 Col 5, 6, 7, 8

2. Glycerol stock patch plate overnight

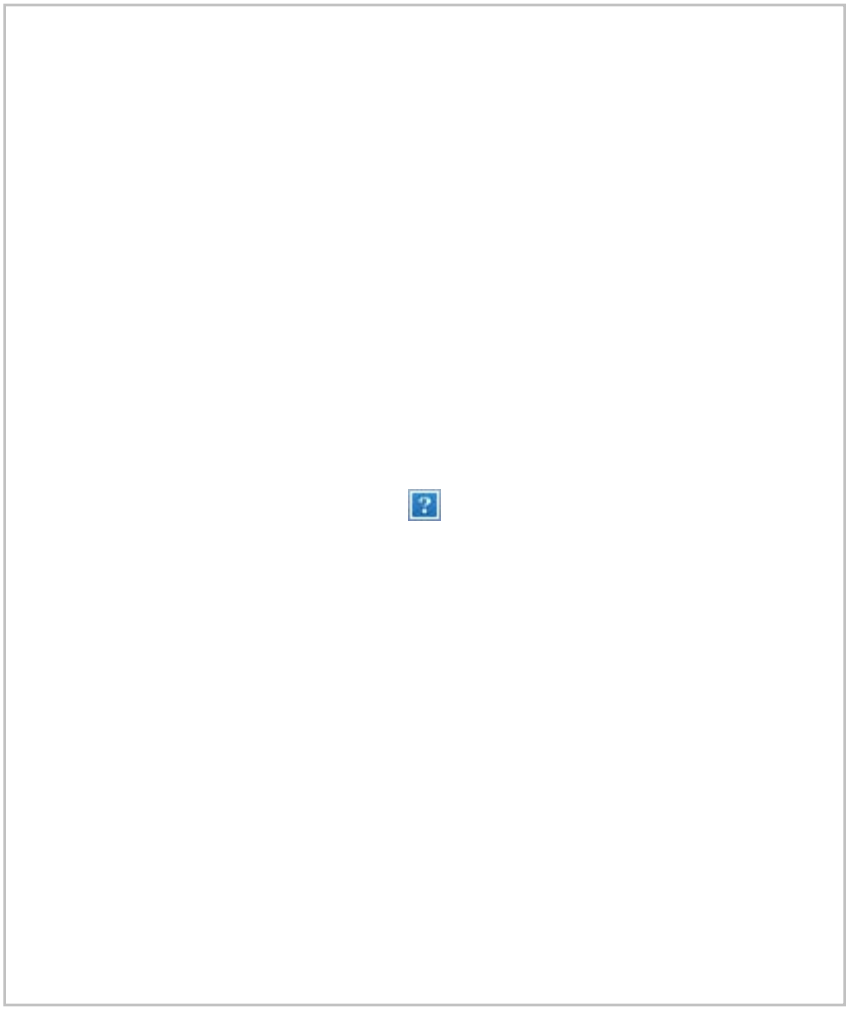
- Created glycerol stock
- New ***yGEM128***!!!
  - CD008DB pTEF1(m10)-BAR1

3. Run gel for Re-PCR pTEF1 m3, m6, m7

- Ran gel
- Lanes:

  1. 2log DNA Ladder
  2. pTEF1(m3)
  3. pTEF1(m6)
  4. pTEF1(m7)
- **m3** and **m6** amplified properly, no show of m7

- Digested and ran m3 and m6 on gel to extract



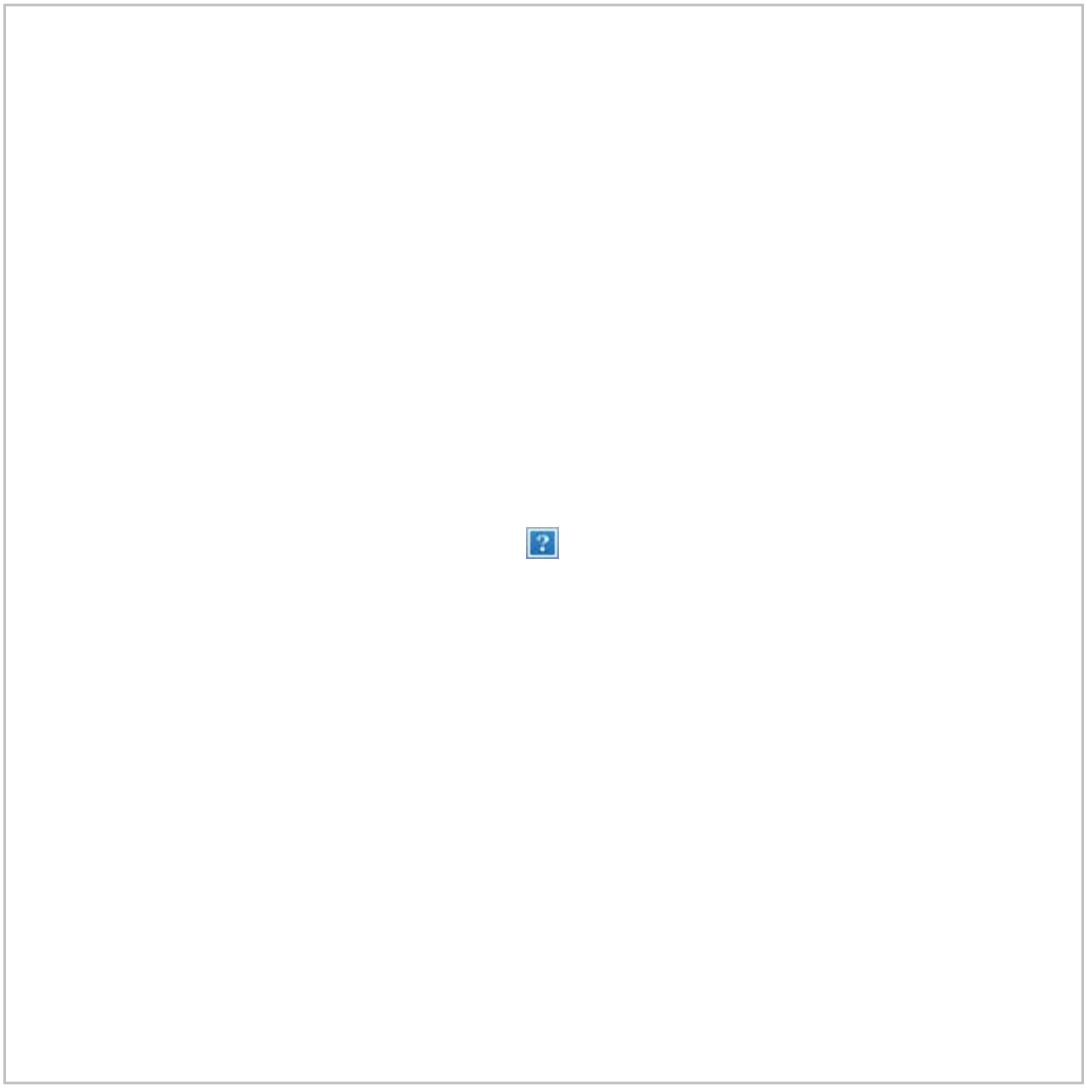
4. Clone pBAR1 + GFP Construct for Parts Registry

- Find backbone with GFP and add pBAR1

7/16/15

1. Prepping pGEM45 and pGEM1

- Digested yesterday, phosphotased treated
- Loaded in gel for extraction
- **BUT these plasmids did not digest properly**



2. Colony PCR Gel again

- Failed, no bands showed up
- Transformations didn't work... why?

7/17/15

1. Gel Extract pGEM45 and pGEM1

- Gel --> plasmids did not **did not digest** (previous day's image)
- For pGEM45:
  - Use 1uL of enzyme for Apa/Xho instead of 0.5uL
  - Digesting 2 hour steps instead of 1 hour
- For pGEM 1:
  - Try digesting pGEM2 as well
  - Use 1uL of enzyme instead of 0.5uL for both pGEM45 and pGEM2
  - Digesting 2 hour steps instead of 1 hour

2. On pBAR1 and pTEF1

- Find PCR products to redigest

3. On pTEF1 mutants (m3, m6, m7)

- Re-digest m3, m6
- Re-PCR m7
- Run on gel to confirm, then PCR clean-up --> digest
- Once complete, assuming correct digest of pGEM45, ligate

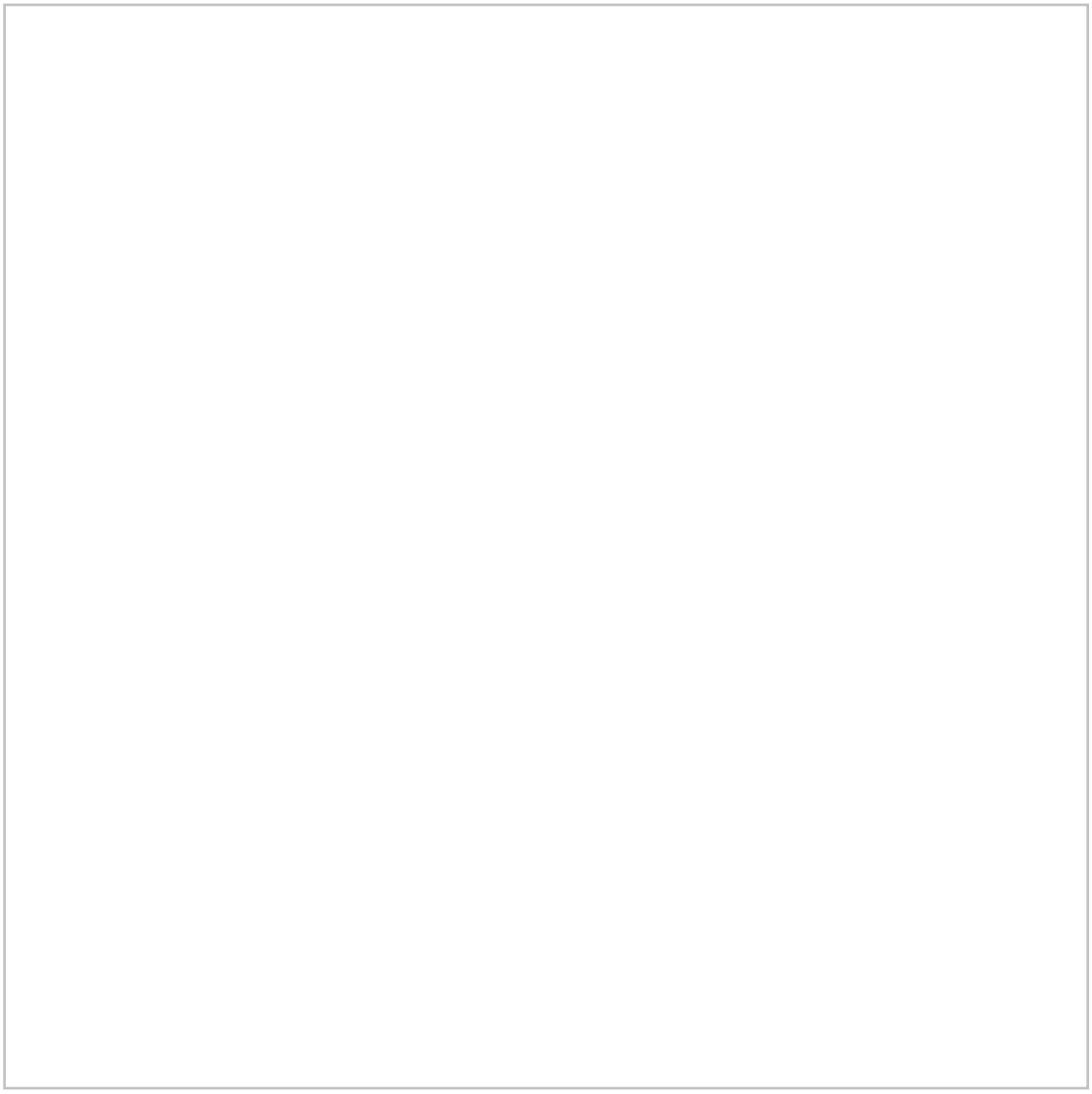
4. pTEF1(m7) PCR Reaction

- Success. Ran gel.

7/20/15

1. Re-PCR m3, m6, m7

- DNA amount was trivial over weekend after gel extraction
- Ran PCR, gel confirmed correct cut length
- Digested with Apa1, Xho1 over night



2. pGEM 1, pGEM 2, pGEM 45

- Digested
- Loaded into gel

3. pBAR1 and pTEF1

- Thinking we need to extract from yeast genomic DNA

7/21/15

1. Gel extracted pGEM 1, 2, 45

- Extracted, purified, nanodropped

Plasmid | Concentration [ng/uL] ------- | :----------: pGEM 1 | 11.31 pGEM 2 | 21.35 pGEM 45 | 38.46

2. Gel extracted pTEF1(m3, m6, m7)

Promoter | Concentration [ng/uL] ---------| :----------: pTEF1 m3 | 64.78 pTEF1 m6 | 88.98 pTEF1 m7 | 83.12

3. Ligation reactions

Material | Mass Required [ng] | Concentration [ng/uL] | Volume [uL] --------- | :----------: |:----------: | -------- pGEM 45 | 50 | 38.46 | 2 pTEF1 m3 | 7.5 | 64.78 | 0.115 pTEF1 m6 | 7.5 | 88.98 | 0.084 pTEF1 m7 | 7.5 | 83.12 | 0.090

pTEF1 dilution:
m3: Add 9 uL H2O + 1 uL m3 --> 6.478 ng/uL --> 1 uL of insert
m6: Add 9 uL H2O + 1 uL m6 --> 8.898 ng/uL --> 1 uL of insert
m7: Add 9 uL H2O + 1 uL m7 --> 8.312 ng/uL --> 1 uL of insert
(labeled as d3, d6, d7)

4. Ligation of m3, m6, m7 to pGEM 45

Material | Negative Control Ligase | 45+promoter Ligase ---------- | :----------: | ---------- | pGEM45 | 2 uL | 2 uL | Dil. promoter | 0 uL | 1 uL | Ligase | 1 uL | 1 uL | Ligase Buffer | 2 uL | 2 uL | H2O | 15 uL | 14 uL | **Total** | 20 uL | 20 uL |

- Later transformed

5. Isolated pBAR1 and pTEF1 from Yeast Genome

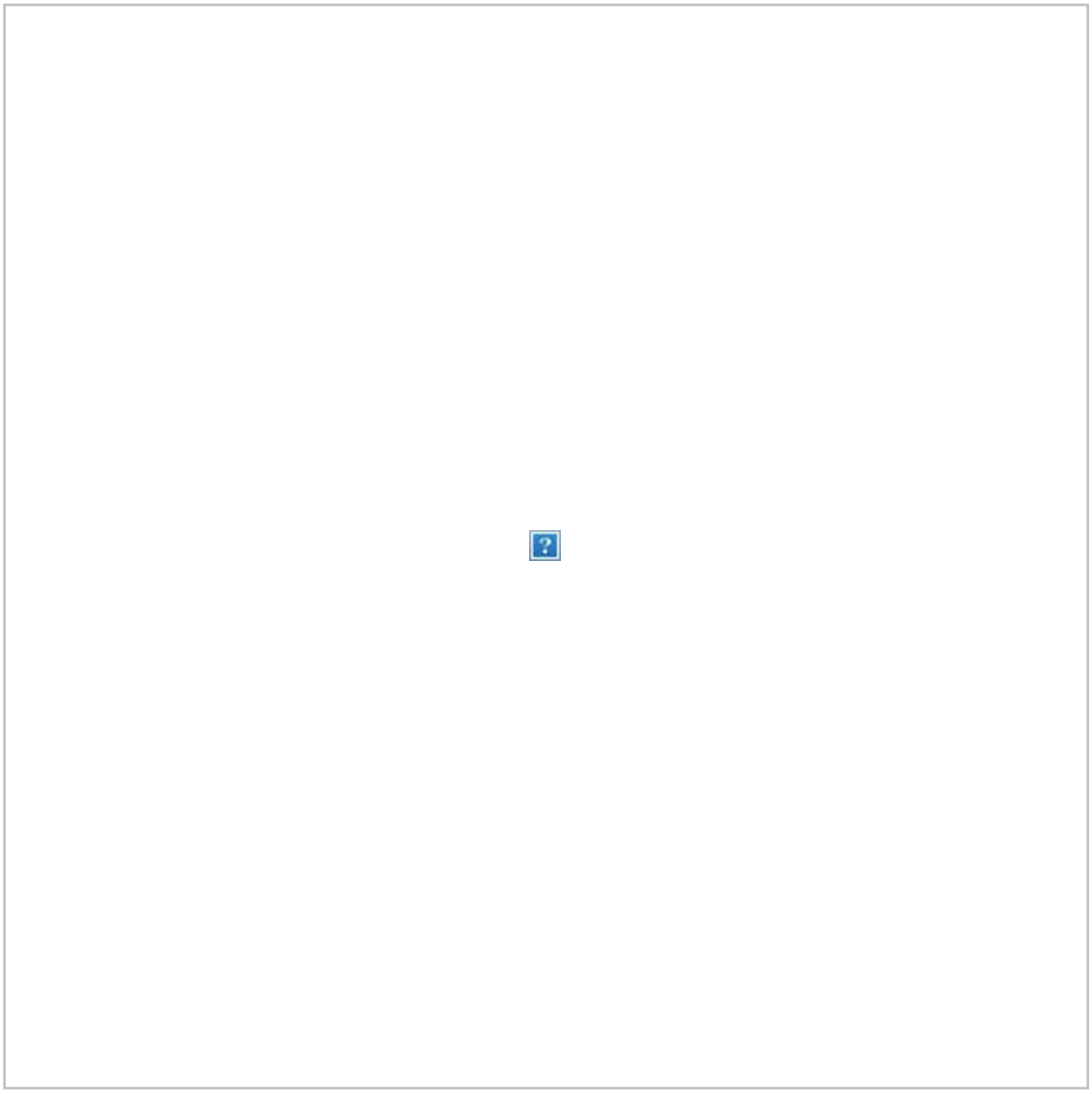
- Running PCR to amplify
- Primers pBAR1 119, 120 diluted
- Primers pTEF1 113, 114 diluted
- Ran on gel, pTEF1 did not amplify, we do have pBAR1

7/22/15

1. pBAR1 Digestion

Lost pBAR1 this morning, PCR'd new pBAR1 - Digest one hour at room with Apa1 (until 5:05pm) - Digest one hour in 37C with Xho1 **Found pBAR1 after pBAR1 was PCR'd.**

```
NOTE: Did not clean up pBAR1 that is being digested.
Cleaning up the new PCR product produced today and digesting that too.
Will be annotated as 'cleaned' or some such label
```



2. PCR pTEF1

- Failed extraction from yeast genome yesterday
- PCRing pGEM 12/17
- Ran gel. pBAR1 amplified correctly. pTEF1 wrong again (GRR.) (to be shown)

3. Colony PCR of m3, m6, m7 + pGEM Transformation

- Have yet to run

7/23/15

1. PCR pTEF1

- Set up PCR for pTEF1 that previously worked (green tube)

2. Colony PCR

- Running gel for col PCR of m3, m6, m7
- 3 lanes m3, 4 lanes m6, 4 lanes m7, 1 lane m3(col4), ladder, 4 neg ctrl

3. O/N Cultures

- Colony PCR worked
- Set up 2 of each mutation O/N liquid culture

4. Setting up new ligations in case PCR doesn't work

- Transformed new m3, m6, m7
- As well as pGEM1 + pBAR1 and pGEM2 + pBAR1

7/24/15

1. Miniprep O/N cultures

- Sent m3, m6, m7 for sequencing

2. Colony PCR of pGEM1, pGEM2 + pBAR1

- Set up and running now.
- Gel looks good

7/27/15

1. Mutant sequencing

- m3, m6, m7 all incorrect
- False positives showed up on our gel... but

2. Re-ligating mutants

- Transformed m3, m6, m7 and negative control

3. pGEM1/2 + pBAR1

- Successful Colony PCR --> O/N culture today

7/28/15

1. pBAR1 + pGEM1/2 Culture

- MiniPrepping then Colony PCRing to negate false positives

2. pTEF1 m3, m6, m7 Transformation Plates

- Plates show no colonies as of 9AM. Checking them later today.
- Checked again at 2PM. No visible m3, m6, m7 colonies.
    - **Ligation was probably unsuccessful with possibly undigested plasmids**
- **Will be trying Gibson Assembly next.** Currently designing primers

3. Preemptive culture of Yeast DB strain

- If pBAR1 sequences correct, we can transform tomorrow

7/29/15

1. Sequencing of pBAR1 + GFP + pSV606 = CORRECT

- Labeled MiniPrepped DNA as **pGEM 51**
- Transforming into yeast

2. Transforming GFP+pBAR1 into yeast

```
Before Transforming:
1. Take 250 uL of overnight stock
2. Put into glass culture tube, add 4.75 mL of YPD
3. Incubate in 30C for 3 hours
4. PME Digest (below) (1 hour)
5. Prep ssDNA (20 min)

PME Digest:
- 2000ng DNA (pTEF1(m10))
- 0.5uL of PME1 enzyme
- 1 uL CutSmart
- Add H2O up to 10uL
- Incubate for 1 hour at 37C

Salmon Sperm DNA Prep:
- Take tube from freezer
- Boil (95C) in PCR machine for 10 min
- Put on ice for ten minutes
```

3. Gibson Assembling pTEF1 mutants + BAR1 later today

7/30/15

1. Digesting pGEM45 for Gibson prep

- Added:
  - 10 uL pGEM 45
  - 7.5 uL H2O
  - 2 uL CutSmart Buffer
  - 0.5 uL Xho1
- Incubate for 1 hour at 37C

2. Gel extract pTEF1 + m3 + m6 + m7

7/31/15

1. Check transformed GFP/BAR1 Yeast Colonies

- Low confluency, will redo transformation just in case

2. Gibson Assembly

- Constructed pGEM45 + m3, m6, m7 and pTEF1 wild type

3. Yeast Transformation

- Transformed pGEM 51 (BAR1 + GFP) into yeast
- Colonies have appeared

8/3/15

1. Col PCR pBAR1 + pGEM 51

- **Primers**: 158 159
  - **417bp** product

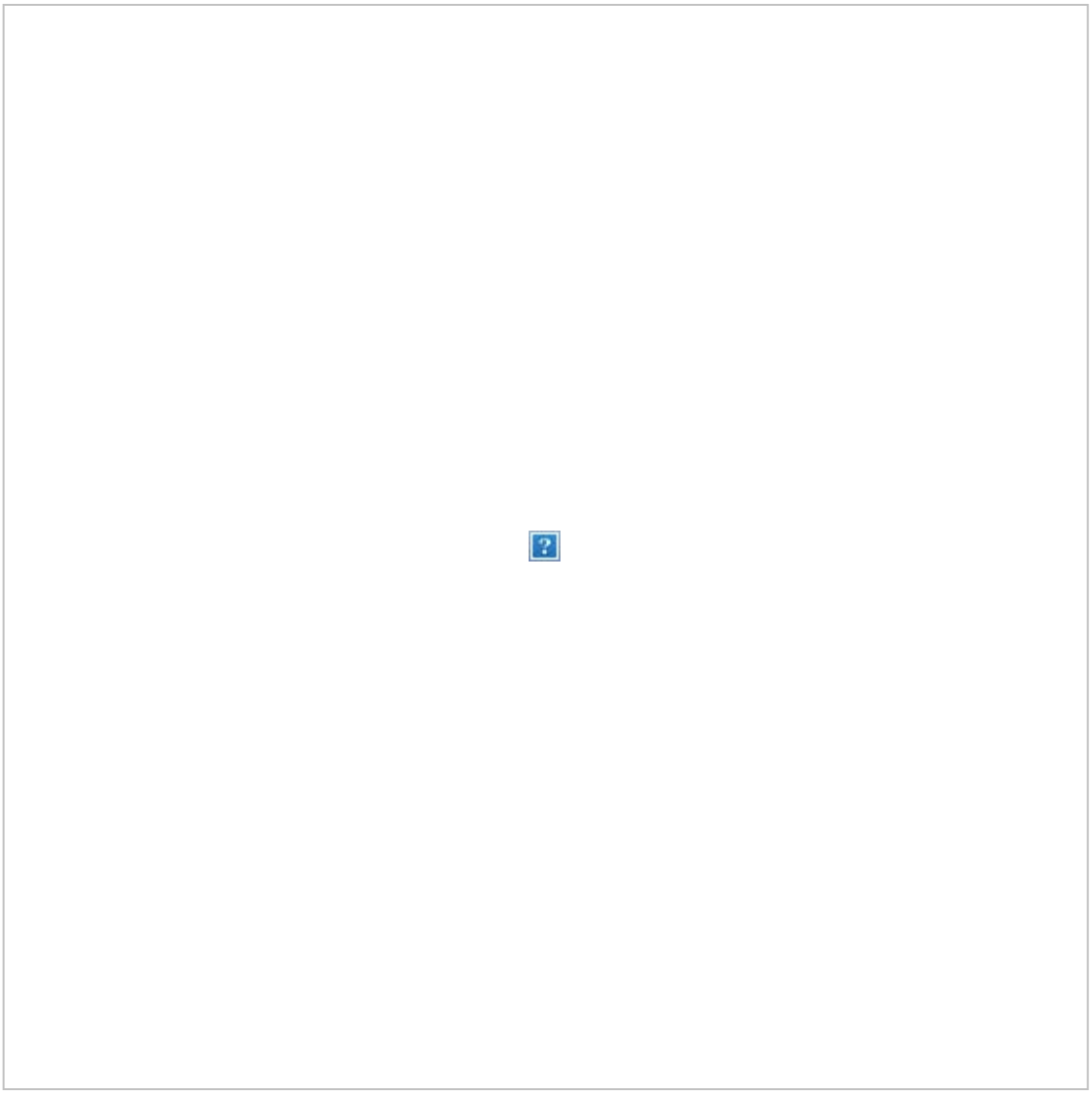
2. Redigesting pGEM45 plasmid

- 1 uL of Apa1
- 1 uL of Xho1

8/4/15

1. Running gel of Colony PCR of pBAR1+pGEM51

- No bands?
- FALSE NEGATIVE --> Everything's great. Got pBAR1+GFP --> **yGEM 132**



2. Colony PCR of Gibson Assembly (pTEF1, m3, m6, m7)

- Ran PCR with only primers for pTEF1 promoters
    - Will only cut promoter out which could result in false positives

3. Colony PCR w/promoter FW primer and BAR1 REV primer

- Need to use FW primer for Promoter and REV for BAR1 (93)
- No bands showed up, earlier run (above) was false positive

FLOW CYTOMETRY 'HOW-TO'

Always leave on Standby Mode. Only two buttons: "Run" and "Standby"

- Controls liquid flow
- Pick up box to check liquid is in there. Never run out.
    - Alarm button turns off alarm that goes off when waste is full
    - Waste has **bleach** so pour slowly into sink to prevent splash
    - Add some bleach to waste bucket if you ever empty it
- Run "Clean Plate"
    - Runs bleach and water through system to clean out system
    - Always run one BEFORE and AFTER
    - Login is **biobricks**
- Double click experiment folder --> open book
    - New Plate --> screen set up 96 well plate
    - Select "Daily Clean Plate"
        - 4 wells cleaning solution, 4 wells red(?) solution
        - 250uL of 10% bleach in A1-4
        - 250uL of H2O in B1-4
    - Keep this plate for AFTER clean run
- Once clean plate in and lid is on, highlight wells you want
    - Acquisition dashboard --> run wells
    - View --> worksheet --> view data
- Mixing volume = half the volume of the sample it is taking
- Run one well first
- Then run the whole plate

8/5/15

1. Gibson Assembly of pTEF1, m3, m6, m7

- Stuff and incubating

8/6/15

1. Colony PCR'd Gibson Assembled cells

- It was awesome! Everything had at least 2 positives
- Sending for sequencing tomorrow

8/7/15

1. MiniPrepping Positive Gibsons

- Sent two of each for Sequencing

2. PME Digest of pGEM34

- Adding BFP to BAR1 constructs
- Digesting pGEM34 (pAga+BFP) to add later

```
PME Digest:
- 2000ng DNA
- 0.5uL/mg of PME1 enzyme
- 1 uL CutSmart
- Add H2O up to 10uL
- Incubate for 1 hour at 37C
```

- For PME: 3 uL of pGEM34 (500ng/uL), 0.5uL PME, 1uL CutSmart, 5.5uL H2O

3. Transforming into yeast

- Adding pGEM34 (BFP) to CB008DB
- yGEM128 --> pTEF1(m10)+BAR1+pJW608

8/10/15

1. Sequencing for pTEF1, m3, m6, m7

- Sequences all correct!!!
- New pGEMs = PARTYYYY.

New pGEMs

Name | Components ------- | ---------- pGEM 58 | pTEF1 + BAR1 + pJW608 pGEM 59 | pTEF1(m3) + BAR1 + pJW608 pGEM 60 | pTEF1(m6) + BAR1 + pJW608 pGEM 61 | pTEF1(m7) + BAR1 + pJW608

2. Do stuff with BAR1 and BFP

3. Transform

- Correct pGEM sequence --pGEMs--> transform into yeast

8/11/15

1. Set up stuff for Flow

- pGEM 34, 133, 134, mix of 34 and 134
- Ran with BFP BAR1 strains, will need a higher promoter

2. Gibson-ing pGEM1 and pBAR1

3. Replica Plated pGEM 58/59/60/61

- Originally on YPD, replica'd onto YPD + Nat

8/12/15

1. Colony PCR pGEM1 + pBAR1

- Use dif FW/REV primers to avoid false positives?
- Primers:
  - pBAR1 (350) --> FW 119
  - GFP (720bp) --> REV 73(?)
- Look for band just over 1kb

2. Colony PCR for yeast

- Wait another day, colonies still growing

3. Made O/N Cultures

- Synthesized pBAR1 + pGEM1

8/13/15

1. PCR pTEF1(m10)

- Kara gave us new primers --> leaves overhangs for Gibson
- Doing this to have a HIGH expression promoter to put in front of BFP
- Gibson-ing later today

2.Colony PCR for pGEM 58/59/60/61 Transformations

- Made patch plate, added cells to 50uL of Zymolase, incubating
- Run Colony PCR with 91 and 87

3. Crazy stuff with pBAR1

- Resending in miniprepped pGEM1/pBAR1 and pGEM2/pBAR1 for sequencing

4. Gibsoning

- PCR'd pBAR1 with proper primers
- Digesting and gel extracting

PCR GEL: First 4 Lanes: pTEF1 Next 5 Lanes: m3 Next 4 Lanes: m6 Next 2 Lanes: m7 Next 3 lanes: pTEF1, m3, m6

8/14/15

1. Sequencing for pBAR1

- pBAR1 + GFP
- All correct!!

2. Gibson'd pTEF1(m10)+BFP

- Plated in drawer over weekend

8/17/15

From Erika's Notebook:

8/18/15

1. Coding for website

- Formating homepage
- Made toolbar static and properly formatted it

2. Modeling

- Got a pretty good working model with two cells communicating
- Need to google reasonable parameters to plug into equations
- Will then vary BAR1/Dox/n cells

8/19/15

1. Who's a code monkey?

- I'm a code monkey

2. Working on MATLAB scripts from last year

- Determine how FACS data was extracted from CSV files
- Then construct graphing algorithm

8/20/15

1. Who's a code monkey?

- I guess that's me again today.

2. MATLAB Data Analysis

- Debugged last year's code

Successful code:
"Import*pTEF1rtTA*pTETGFP_v*Doxy*FITCFromCSV"

- Uses:
    - 120804 pTEF1 rtTA pTET GFP ROUND 3'
    - 140729 pTEF1 rtTA pTET GFP DB ROUND 2'
- Returns:
    - One graph for each ROUND
    - Dose response curve for [DOXY]

"Import*pTEF1GFP*FITFromCSV"

- Uses:
    - 140627 AFRP GFP ROUND 2',
    - '140627 AFRP GFP ROUND 3',
    - '140630 AFRP GFP ROUND 4',
    - '140702 AFRP GFP ROUND 5',
    - '140710 AFRP GFP ROUND 6',
    - '140710 AFRP GFP DB ROUND 1',
    - '140711 AFRP GFP DB ROUND 2',
    - '140715 AFRP GFP DB ROUND 3',
    - '140722 AFRP GFP DB ROUND 4',

STATUS:

- First files graph vs DOXY. Shipping this with instructions today.

- Second files graph vs ALPHA. We will only need this with BAR1 and we are currently still having issues with it.
    - Erroring because only supplies FITC but not SD and other columns??

GOAL: - Create a shippable version of "Import*Data*Dox" that is user friendly for iGEMers to use for their FACS data